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Genetic and Metabolic Variability between Two Subspecies of *Chamaeleo chamaeleon* (Reptilia: Chamaeleonidae) in Egypt

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Abstract

The degree of variability between two subspecies of *Chamaeleo chamaeleon*; *C. chamaeleon chamaeleon* inhabiting El-Dabaa (MarsaMatrouh) and *C. chamaeleon musae* inhabiting El-Arish (North Sinai) of Egypt was investigated in this study using polyacrylamide gel electrophoreses for Lactate dehydrogenase (*Ldh*) and Alfa-esterase (*α-Est*) isoenzymes. Total lipids and proteins of liver and muscle tissues in both species were analyzed. Three *Ldh* isoforms were recorded for both subspecies and the activity. Rate of flow (RF) of *Ldh-1* seemed to be higher in *C. chamaeleon chamaeleon* than in *C. chamaeleon musae*. This high activity could be supported by the significant increase in the total lipids and proteins in liver and muscle tissues of this species. It may thus be reasonable to suppose that *C. chamaeleon chamaeleon* is more active, energetic and adaptable in its habitat than *C. chamaeleon musae*. The *α-Est* showed four fractions in both subspecies. The null variations in the activity of *α-Ests* in the studied tissue may indicate, to some extent, the safety of the diet applied to both subspecies of chameleons.

Keywords

Electrophoreses, Physiological Ecology, Chamaeleonidae, Isoenzymes, Lipids, Proteins

1. Introduction

East Africa has a diverse chameleon fauna with over 50 species described to date. These species are mostly regional endemics restricted to highlands areas and adapted to cooler and higher rainfall environments [1]. The Common Chameleon, *Chamaeleo chamaeleon*, belongs to the family Chamaeleonidae. These Old World lizards possess unique features that make them easily distinguishable from other lizards. Included in the features are

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their zygodactyl feet and laterally compressed body. Their adjacent digits are fused on each foot, forming opposing grasping pads [2].

Chamaeleonidae is composed of six genera, which include *Bradypodion*, *Brookesia*, *Calumma*, *Chamaeleo*, *Furcife*, and *Rhampholeon* [3]. The Common Chameleon features the broadest distribution of all chameleon species, found from Morocco and the southern Iberian Peninsula over the whole of North Africa, to the Near East, Turkey, Cyprus and Southern Arabia [4]. The genus *Chamaeleo* contains 4 recognized subspecies: *C. c. chamaeleon*, *C. c. musae*, *C. c. orientalis*, and *C. c. rectricrista*. The subspecies; *C. c. chamaeleon* and *C. c. musae*are allocated from North Africa, Middle East, Morocco, Algeria, Tunisia, Libya, Egypt, Israel, Palestine, Jordan, Western Sahara, Saudi Arabia, Yemen, Lebanon, Syria, Iraq, and Iran [5].

Isoenzymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. *Ldh*sisozymes are systems very suitable for our examination of several metabolic, genetic, ecological features, and very useful in systematic studies [6]. *Ldhs* as a kind of hydrogen transfer enzyme, catalyze the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD)+ as hydrogen acceptor, which constitutes the final step in the metabolic chain of anaerobic glycolysis. Esterase isoenzymes (*Est*s), as one of the lipid-hydrolyzing enzymes, possess high significance in genetics and toxicology [7].

Electrophoresis is a versatile biochemical technique to detect genetic variation between subspecies depending on the migration of the charged molecules, such as isoenzymes, in an electric field [8]. Thus the present study aims to investigate the patterns of inter-specific genetic and biochemical variations between two subspecies of *C. chamaeleon* (*C. chamaeleon chamaeleon* and *C. c. musae*) in the coastal and Sinai deserts of Egypt.

2. Materials and Methods

2.1. Taxon Sampling and Study Area

A total of 10 samples from 2 Egyptian subspecies of chamaeleonid lizards; *C. c. chamaeleon* and *C. c. musae* were collected from El-Dabaa (Marsa Matrouh) and El-Arish (North Sinai) respectively [31˚01' 37.49"N 28˚26' 8.48"E and 31˚07'55.53"N 33˚48'11.79"E respectively] (**Figure 1**).

2.2. Sample Preparation and Isoenzyme Assay

Tissue samples of liver and heart were removed, immediately taken to the lab, and stored at −80˚C for further laboratory use. For isoenzyme extraction, approximately 0.5 g of tissue was homogenized in 10 mL saline solution (PBS, pH = 6.8), using a manual Homogenizer. The homogenates were centrifuged at 5000 rpm for 10 minutes and the supernatants were kept at −20˚C until use. The enzymes; Alfa-esterase (*α-Est*) in heart and Lactate dehydrogenase (*Ldh*) in liver supernatants were separated by discontinuous polyacrylamide gel electrophoresis [9] [10].

Electrophoresis was carried out conveniently in discontinuous polyacrylamide gels. An amount of 50 µl of the clear supernatant of the liver and muscle homogenate of each sample was mixed with 20 µl of protein dye (1% bromophenol blue) and 20 µl of 2% sucrose. Thirty µl of the mixture per gel slot were applied per each sample for isoenzymes electrophoresis. After electrophoresis, the gel was transferred into a staining solution (50 - 70 ml) according to [11], which was then replaced by a destaining mixture of methanol, acetic acid and water (5:1:5 v/v/v). A potential gradient of high voltage electrode [(20 v/cm), anode] across the gel was applied for 4 h at 8˚C to separate the enzymes.

For *Ldh* and the electrophoresis after it, the gel was soaked in 100 mL of 0.2 M Tris-HCl (pH 8.0) containing 30 mg NBT, 25 mg EDTA, 50 mg NAD, 10 mg L-Lactic acid and 2 mg PMS. There was 0.05 M Tris-HCl pH 8.5 prepared by dissolving 0.605 g Tris in 50 mL distilled water. The pH was adjusted to 8.5 by HCl. Then the solution was completed to 100 ml by distilled water [12].

Regarding *α-Est*, after the electrophoresis, the gel was soaked in 0.5 M borate buffer (pH 4.1) for 90 minutes at 4˚C. This procedure lowers the pH of the gel from 8.8 to about 7 at which the reaction proceeds readily. The low temperature minimizes diffusion of the protein within the gel. The gel then was rinsed rapidly in two changes of double distilled water. The gel was stained for esterase activity by incubation at 37˚C in a substrate solution of 100 mg *α*-naphthyl acetate and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 [13].

After the appearance of the enzyme bands, the reaction was stopped by washing the gel two or three times

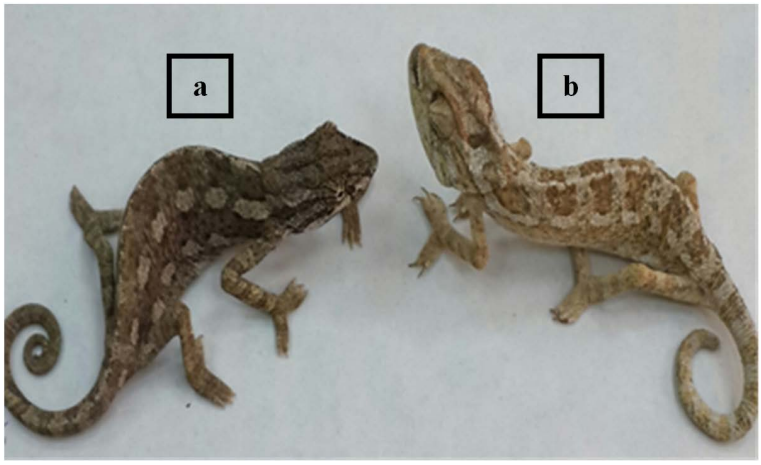


Figure 1. Photos of *C. c. chamaeleon* (a) and *C. c. musae* (b) inhabiting El-Dabaa (MarsaMatrouh) and El-Arish (North Sinai) respectively.

with tap water. This was followed by adding the fixative solution, which consists of ethanol and 20% glacial acetic acid (9:11 v/v). The gel was kept in the fixative solution for 24 hours and then photographed.

2.3. Metabolic Study

Immediately after collection, chamaeleons were weighed in grams (g) to the nearest 0.01 - 0.1 g and dissected. Pieces of liver and thigh muscles were removed and immediately weighed in grams (g) to the nearest 0.01 g. They were stored frozen at −20˚C till use. Livers and thigh muscles were processed to estimate the total lipids and total proteins according to the method of [14] and [15] respectively, using a kit of Biodiagnostics Company.

3. Statistics

All gels were scanned using Gel Doc-2001 Bio-Rad system. For isoenzymes, the bands of enzyme activity were designated according to the system nomenclature proposed by [16].

An abbreviation which corresponds to the name of the enzyme was designated to each locus. When multiple loci were involved, the fastest anodal protein band was designated as Locus One, the next as Locus Two and so on. Student t-test in the PASW package v. 20 was used to calculate the significance difference of total lipids and total proteins within and between species.

4. Results and Discussion

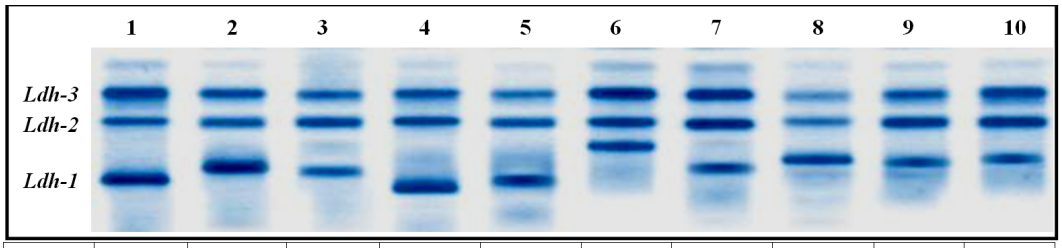
Three *Ldh* isoforms were recorded for both subspecies of *C. chanaeleon*. The activity of *Ldh-1*isoform seemed to be higher in *C. c. chanaeleon* than in *C. c. musae*because greater thickness, density, and the rate of flow (RF) of the band in *C. c. chanaeleon*was observed (**Figure 2**). *Ldh*sisozymes are systems that both very suitable systems for studying several metabolic, genetic, ecological features, and are very useful in systematic studies [6]. *Ldhs* are ahydrogen transfer enzymes that catalyze the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD)+ as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favours the reverse reaction, namely the reduction of pyruvate (P) to lactate (L):

$$\text{L-lactate} + \text{NAD}^+ \underset{\text{pH 7.4 - 7.8}}{\overset{\text{LDH, pH 8.8 - 9.8}}{\leftrightarrows}} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

Due to its ability to produce NADH, this enzyme is thought to be a key enzyme in lipid biosynthesis. The apparent increase in the activity of *Ldh* in liver tissues of *C. c. chanaeleon*, in the present study, could be supported by the significant increase in the total lipids and proteins in liver and muscle tissues of this subspecies. This subspecies is also shown to be fattier than *C. c. musae*. It may thus be reasonable to suppose that *C. c. chanaeleon* is more active, energetic, and adaptable in its habitat than *C. c. musae*.

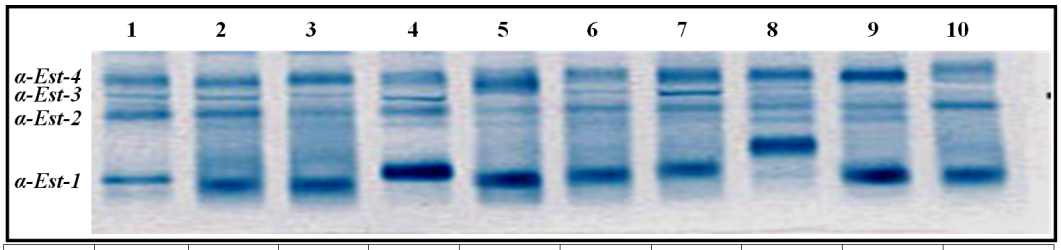
The *α-Est* showed four fractions in both subspecies of chamaeleons except in the case of the last sample in *C. c. musae*, which showed three fractions with absence of the third fraction. The fractions of the two subspecies of chamaeleons were the same in terms of density and thickness. And their rates of flow were nearly the same (**Figure 3**). Esterases are used as bioindicators to measure the toxic potency of pesticide residues usually applied in the field [6] [7]. The null variations in the activity of *α-Ests* in the studied tissue may indicate, to some extent, the safety of the diet applied to both subspecies of chameleons.

Table 1 shows the mean and standard error values of total lipids and proteins in liver and muscle tissues of



Bands	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF
3	0.1	0.11	0.12	0.11	0.12	0.11	0.12	0.13	0.12	0.1
2	0.35	0.36	0.36	0.35	0.36	0.36	0.37	0.35	0.36	0.36
1	0.85	0.75	0.78	0.93	0.86	0.56	0.75	0.68	0.7	0.67

Figure 2. The electrophoretic profile of *Ldh* isoenzymes in liver tissues. Lanes are as follow: 1 - 5 (*C. c. chanaeleon*), 6 - 10 (*C. c. musae*).



Bands	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF
4	0.073	0.091	0.073	0.064	0.11	0.036	0.045	0.036	0.045	0.25
3	0.18	0.2	0.17	0.2	0.28	0.16	0.17	0.25	0.25	-
2	0.32	0.3	0.3	0.29	0.37	0.26	0.26	0.34	0.33	0.55
1	0.76	0.79	0.79	0.72	0.74	0.72	0.68	0.53	0.73	0.72

Figure 3. The electrophoretic profile of *α-Est* isoenzymes in the studied heart tissues. Lanes are as follow: 1 - 5 (*C. c. chanaeleon*), 6 - 10 (*C. c. musae*).

Table 1. Comparison of total lipids and total proteins in liver and muscle tissues of *C. c. chamaeleon* and *C. c. musae*. Data are expressed as mean ± standard error. Number of individuals between parentheses.

Parameters	*C. c. chamaeleon*	*C. c. musae*	*t-test*
Liver total lipids (mg/100 mg)	19.2807 ± 7.04712 (5)	8.4262 ± 1.40782 (5)	1.981*
Thigh muscle total lipids (mg/100 mg)	4.5158 ± 1.05328 (5)	4.1712 ± 1.47154 (5)	5.080*
t-test	2.768*	5.277*	
Liver total proteins (mg/100 mg)	153.7200 ± 48.56457 (5)	79.3716 ± 14.09141 (5)	1.353*
Thigh muscle total proteins (mg/100 mg)	5.4376 ± 1.76793 (5)	5.1715 ± 2.22419 (5)	4.525*
t-test	2.295*	4.610*	
Body weight (g)	25.0200 ± 0.95677 (5)	24.0250 ± 2.64650 (5)	0.388

*Highly significant at $P < 0.05$.

both subspecies of chamaeleons. By comparing the total lipids and total proteins of liver and muscle tissues in the two *C. chamaeleon*subspecies, one canfindasignificantly bigger increase ($P < 0.05$) of the total lipids and total proteins in liver and muscle tissues of *C. c. chamaeleon* than in *C. c. musae.* Within each subspecies, total lipids and proteins were significantly higher in liver ($P < 0.05$) tissues than in muscle.

In conclusion, *C. c. chamaeleon* acquired high physiological performance and activity than *C. c. musae*. Between the two, isoenzyme expression in the former species was higher than in the latter. The accumulation of total lipids and proteins were also significantly higher in the former subspecies than in the latter.

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